PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 3259PTWO/er	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/EP2004/050538	International filing date *(day/month/year)* 16.04.2004	Priority date *(day/month/year)* 18.04.2003
International Patent Classification (IPC) or national classification and IPC C07J31/00, C07J9/00		
Applicant ERREGIERRE S.P.A.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15.02.2005	12.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Watchorn, P Telephone No. +31 70 340-2207

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/EP2004/050538

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-11	as originally filed
Claims, Numbers	
1-24	received on 15.02.2005 with letter of 15.02.2005
Drawings, Sheets	
1/12-12/12	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-24
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V.

The following documents constitutes the closest state of the art with regard to the presently claimed subject matter:

D1 : WO 97/18816 A (CHILDRENS HOSP MEDICAL CENTER) 29 May 1997 (1997-05-29)

The following documents are also relevant for the assessment of the inventive step of the presently claimed subject matter:

D2 : US 4 565 811 A (DI SCHIENA MICHELE G) 21 January 1986 (1986-01-21)

D3 : BANDIERA T ET AL: "A CONVENIENT PROCEDURE FOR THE SYNTHESIS OF URSODEOXYCHOLIC ACID SULFATED DERIVATIVES" SYNTHETIC COMMUNICATIONS, MARCEL DEKKER, INC., BASEL, CH, vol. 17, no. 9, 1987, pages 1111-1117, XP000564355 ISSN: 0039-7911

D4 : DATABASE CA [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; TOZAWA, MICHIKO: "Sulfation of steroids with sulfamic acid" XP002268885 retrieved from STN Database accession no. 73:99091

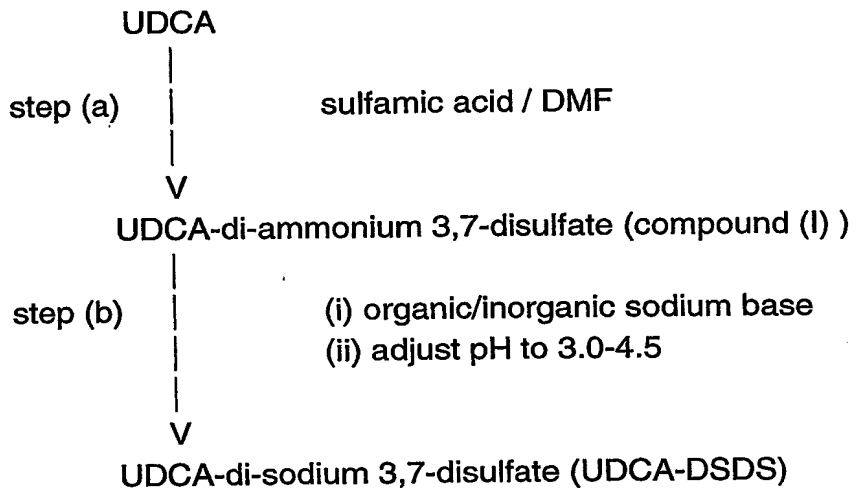
D5 : US 3 271 250 A (KEN-ICHI KANAZAWA ET AL) 6 September 1966 (1966-09-06)

D6 : US 3 836 527 A (FREISBERG K ET AL) 17 September 1974 (1974-09-17)

D7 : DE 196 42 875 A (HENKEL KGAA) 23 April 1998 (1998-04-23)

Novelty (Article 33(2) PCT)

The subject matter of claim 1 relates to a process for the production of ursodeoxycholic acid (UDCA) - di-sodium 3,7-disulfate (referred to hereinafter as UDCA-DSDS). The process of claim 1 is divided up into two steps, step (a) and step (b):



In this regard it is noted that document D1 discloses a process for the production of the same final product (see page 26, last paragraph), whereby the corresponding step (a) in D1 is carried out using sulfuryl chloride ($HOSO_2$-Cl) in the presence of pyridine (which forms an intermediary SO_3:Pyridine complex which acts as the sulfation agent). This leads to the UDCA-3,7-disulfate either in its acidic form or as the corresponding pyridinium salt as an intermediate, which is then, as in claim 1, salified with a sodium base to produce the corresponding di-sodium salt of the 3,7-disulfate (UDCA-DSDS).Consequently, the subject matter of claim 1 and claims 2-19 dependent thereon is novel according to Article 33(2) PCT.

The subject matter of independent claim 20 relates to the intermediate compound - UDCA-di-ammonium 3,7-disulfate (compound (I)). Since the process of the closest state of the art does not result in an intermediate ammonium salt (but rather the intermediate in D1 is the acidic form of the 3,7-disulfate or as the corresponding pyridinium salt), this bi-ammonium salt of UDCA-3,7-disulfate is novel according to Article 33(2) PCT as is the process for producing this intermediate according to independent claim 21 and claims 22-24 dependent thereon (the process of claim 21 is in fact the same as step (a) of claim 1, but claimed independently of step (b)).

Documents D2 and D3 also disclose the production of UDCA-3,7-disulfate sodium salts also by means of the SO_3:Pyridine complex as the sulfation agent and subsequent salification with sodium bases, however, these two documents disclose the production of the tri-sodium salt (where the C_{17}-carboxylic acid group of the UDCA bile acid is also salified). Consequently, the claimed process (both of claims 1 and 21) is novel over the disclosures of these documents (see D2, example 1 and D3, page 1112 last paragraph - page 1113, paragraph 2, table 1) in that the initial sulfation is carried out using sulfamic acid and DMF in claims 1 and 21, whereas in D2 and D3, this is carried out using SO_3:Pyridine complex which does not yield the di-ammonium salts of claim 20, but rather the corresponding acidic form of the di-sulfate or the corresponding pyridinium salt.

Inventive Step (Article 33(3) PCT)

The problem to be solved by the currently claimed subject matter is the provision of an improved process and intermediates for the production of UDCA-DSDS, which in particular makes use of less toxic reagents (see page 2, paragraph 1 of the description). In this regard it is noted that the use of sulfamic acid for the sulfation of steroid-hydroxyl groups is known from documents D4-D7 (see D4, abstract; D5, column 5, lines 33-44; D6, examples 1,2; D7, claim 1). It is also noted that the subsequent salification of the sulfates so-produced is also known from documents D5-D7 (see above mentioned passages).

The sterols sulfated in D4-D7 are not bile acid derivatives and so these documents do not prejudice the novelty of claims 1-24. They do clearly indicate that sulfamic acid is a viable alternative to SO_3:pyridine, indeed in document D4, the use of sulfamic acid:pyridine gave a better yield than SO_3:pyridine in a mono-sulfation reaction (see abstract). Furthermore, it is also generally known that sulfamic acid is less toxic and hazardous to handle that SO_3:pyridine and, in particular, the reagents needed to produce SO_3:pyridine (namely $HOSO_2$-Cl (see D1, page 26, last paragraph).

Notwithstanding the above, the following is noted. The process of sulfation carried out in documents D4 and D6 makes use of sulfamic acid in the presence of pyridine, whereas the presently claimed process makes use of sulfamic acid in the presence of DMF. In D6, example 2, the sulfation of more than one steroid hydroxy group is achieved using sulfamic acid and pyridine wherein the sulfamic acid was used in a 10x molar excess of sulfamic

acid (in the presence of pyridine) relative to the sterol in order to sulfate three hydroxy groups. However, in the application, the applicant achieves 3,7-di-sulfation using sulfamic acid in the presence of DMF in almost stoichiometric amounts of sulfamic acid (see example 1 of the present application where 278 moles of sulfamic acid in DMF and 127 moles of UDCA sucessfully achieved a 93.7% yield of 3,7-disulfate). Furthermore, although D5 indicates that 3-sulfation of sterols can be achieved using sulfamic acid and DMF (see D5, col 5, lines 33-44) and this was done with a stoichiometric amount of sulfamic acid (10 mmol of sulfamic acid to 10 mmol of 3ß-OH-sterol) this was a mono-sulfation of the relatively reactive 3ß-OH group and does not provide any information about how efficient the use of sulfamic acid and DMF would be in the sulfation of multiple OH groups - which in particular given the teaching of example 2 of D6, the skilled person would expect to require a large excess of sulfamic acid. The applicant has on the contrary, used sulfamic acid and DMF and found that the di-sulfation can be achieved using only a moderate excess and give very good yields (93.7% - see example 1 of the present application). Furthermore, the di-sulfated di-ammonium products produced by the claimed process (as opposed to the pyridinium salts produced in the process of D1) are easier to isolate from the reaction -medium - in D1 the di-sodium salt had to be isolated by treatment with an absoprtion cartridge and in D3 the di-sulfate was isolated using a silica-gel column for flash chromatography. For the presently claimed process, the product could be isolated simply by crystallisation (from acetone - see examples 1 and 2 of the present application). Consequently, the presently claimed process of both claim 1 and claim 21 and claims 2-19 and 22-24 dependent thereon respectively (which both use sulfamic acid in the presence of DMF to di-sulfate UDCA) is an unobvious solution to the problem indicated above and is as such inventive according to Article 33(3) PCT.

Furthermore, since the subject matter of claim 1 is inventive, then the intermediate (compound (I)) as specified in claim 20, which is inevitably produced in the course of this inventive reaction is consequently also inventive according to Artoicle 33(3) PCT.

VIII - Statement according to Rule 70.12(ii) PCT

Claim 20 specifies on the first step of the di-sulfation reaction (step (a) - reaction with sulfamic acid and DMF), whereas the earlier claim 1 specifies both step (a) followed by the salification step (b). Consequently, since the broader claim 20 covers the more specific

claim 1, there is no reason for formulating claim 1 as an independent claim according to Rule 6.4 PCT. Claim 20 should be the first independent claim with current claim 1 referring back to it in accordance with Rule 6.4 PCT - this would constitute the most appropriate grouping of the claims according to Rule 6.4(c) PCT.

There is a minor error in the reformulated claim 16, which depends on claim 13. This claim specidies preferred embodiments of claim 15 (stage c') and this reaction stage is not defined in claim 13, but rather in claim 15. Cosequently, this claim should depend on claim 15 instead of claim 13 (Rule 6.4 PCT).

CLAIMS

1. Process for preparing ursodeoxycholic acid di-sodium 3,7-disulfate comprising:
a) reacting ursodeoxycholic acid with sulfamic acid in dimethylformamide to give ursodeoxycholic acid di-ammonium 3,7-disulfate;
b) treating the ursodeoxycholic acid di-ammonium 3,7-disulfate with organic sodium bases or inorganic sodium bases then treating the reaction mixture with an inorganic acid until a pH between 3.0 and 4.5 is reached to give ursodeoxycholic acid di-sodium 3,7-disulfate in solution.

2. Process as claimed in claim 1 wherein the reaction of stage a) is conducted at a temperature between 40°C and 110°C.

3. Process as claimed in claim 2 wherein the temperature is between 80°C and 90°C.

4. Process as claimed in claim 1 wherein ursodeoxycholic acid di-ammonium 3,7-disulfate is separated from the reaction mixture of stage a) by fractional crystallisation with acetone.

5. Process as claimed in claim 1 wherein the inorganic sodium bases in stage b) are chosen from the group consisting of: sodium hydroxide, sodium carbonate and sodium bicarbonate.

6. Process as claimed in claim 1, wherein the organic sodium bases are sodium acetate.

7. Process as claimed in claim 1 wherein in stage b) the treatment of ursodeoxycholic acid di-ammonium 3,7-disulfate with organic sodium bases or inorganic sodium bases is conducted in alcoholic solvent.

8. Process as claimed in claim 7 wherein the alcoholic solvent is chosen from the group consisting of linear or branched lower C1-C4 alcohols or their mixtures.

9. Process as claimed in claim 8 wherein the alcohol is methanol.

10. Process as claimed in claim 1 wherein in stage b) the treatment with organic sodium bases or inorganic sodium bases is conducted at a temperature between -10°C and 30°C.

11. Process as claimed in claim 10 wherein the temperature is between 0°C and 5°C.

12. Process as claimed in claim 1 wherein in stage b) the treatment with organic

sodium bases or inorganic sodium bases is conducted under vacuum.

13. Process as claimed in claim 1 wherein in stage b) the acidification of the reaction mixture after treatment with organic sodium bases or inorganic sodium bases is conducted by treating the reaction mass with an inorganic acid chosen from the group consisting of: hydrochloric acid, sulphuric acid, 85% (w/w) phosphoric acid or their mixtures.

14. Process as claimed in claim 13, wherein the acid is 85% phosphoric acid.

15. Process as claimed in claim 1 also comprising stage c), consisting in the recovery of ursodeoxycholic acid di-sodium 3,7-disulfate from the reaction mixture, said stage comprising: c') removing, by filtration, precipitated inorganic salts formed after acidification treatment and c") precipitating ursodeoxycholic acid di-sodium 3,7-disulfate from the filtrate whereby the solution containing ursodeoxycholic acid di-sodium 3,7-disulfate is concentrated by distillation and the residue is re-dissolved in organic solvent, preferably acetone, to isolate ursodeoxycholic acid di-sodium 3,7-disulfate.

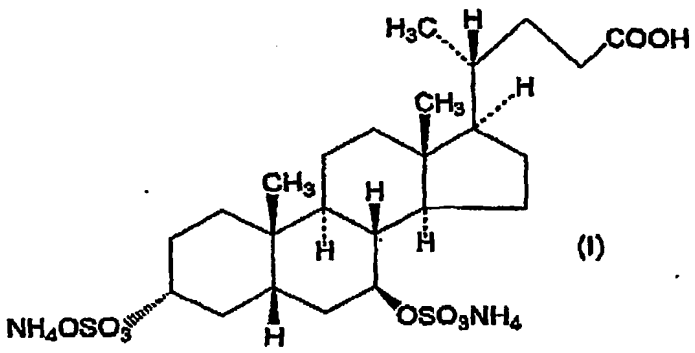
16. Process as claimed in claim 13 wherein in stage c') the filtration to remove the precipitated inorganic salts formed after acidification treatment is facilitated by treating the reaction mixture derived from stage b) with an organic solvent.

17. The process as claimed in claim 16, wherein said organic solvent is acetone.

18. Process as claimed in claim 15 wherein in stage c") the residue obtained by concentrating the solution containing ursodeoxycholic acid di-sodium 3,7-disulfate by distillation is re-dissolved in an organic solvent, at a temperature between 20°C and 70°C, and the suspension thus obtained is then cooled to room temperature and filtered to obtain ursodeoxycholic acid di-sodium 3,7-disulfate as precipitate.

19. The process as claimed in claim 18, wherein said organic solvent is acetone and ursodeoxycholic acid di-sodium 3,7 disulfate is dissolved in said solvent at a temperature comprised between 55° and 65°C.

20. Ursodeoxycholic acid di-ammonium 3,7-disulfate of formula:



(I)

21. Process for synthesising ursodeoxycholic acid di-ammonium 3,7-disulfate comprising reacting ursodeoxycholic acid with sulfamic acid in N,N-dimethylformamide.

22. Process as claimed in claim 21 wherein the reaction with sulfamic acid is conducted at a temperature between 40°C and 110°C.

23. Process as claimed in claim 22 wherein the temperature is between 80°C and 90°C.

24. Process as claimed in claim 21 wherein the ursodeoxycholic acid di-ammonium 3,7-disulfate is separated from the reaction mixture by fractional crystallisation with acetone.